

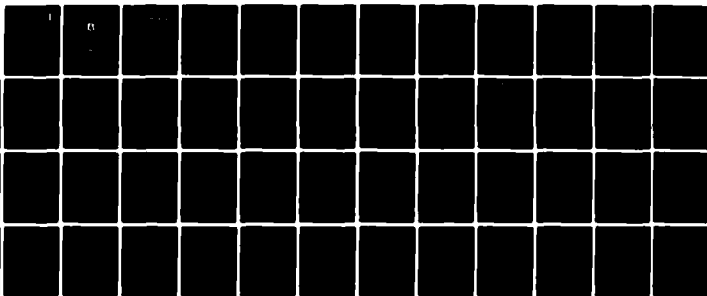
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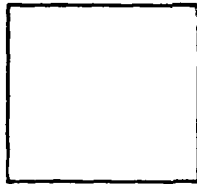
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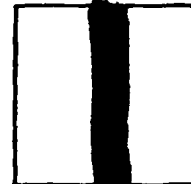
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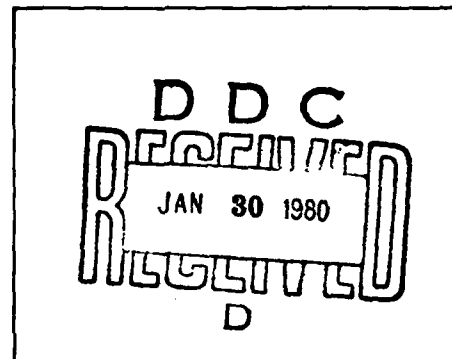
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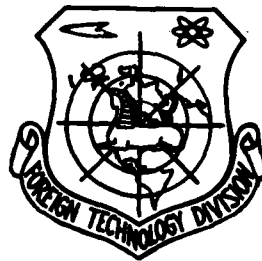
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U. S. BOARD ON GEOGRAPHIC NAMES TRANSLITERATION SYSTEM

Block	Italic	Transliteration	Block	Italic	Transliteration
А а	<i>А а</i>	A, a	Р р	<i>Р р</i>	R, r
Б б	<i>Б б</i>	B, b	С с	<i>С с</i>	S, s
В в	<i>В в</i>	V, v	Т т	<i>Т т</i>	T, t
Г г	<i>Г г</i>	G, g	У у	<i>У у</i>	U, u
Д д	<i>Д д</i>	D, d	Ф ф	<i>Ф ф</i>	F, f
Е е	<i>Е е</i>	Ye, ye; E, e*	Х х	<i>Х х</i>	Kh, kh
Ж ж	<i>Ж ж</i>	Zh, zh	Ц ц	<i>Ц ц</i>	Ts, ts
З з	<i>З з</i>	Z, z	Ч ч	<i>Ч ч</i>	Ch, ch
И и	<i>И и</i>	I, i	Ш ш	<i>Ш ш</i>	Sh, sh
Й й	<i>Й й</i>	Y, y	Щ щ	<i>Щ щ</i>	Shch, shch
К к	<i>К к</i>	K, k	Ъ ъ	<i>Ъ ъ</i>	"
Л л	<i>Л л</i>	L, l	Ы ы	<i>Ы ы</i>	Y, y
М м	<i>М м</i>	M, m	Ь ь	<i>Ь ь</i>	'
Н н	<i>Н н</i>	N, n	Э э	<i>Э э</i>	E, e
О о	<i>О о</i>	O, o	Ю ю	<i>Ю ю</i>	Yu, yu
П п	<i>П п</i>	P, p	Я я	<i>Я я</i>	Ya, ya

*ye initially, after vowels, and after ъ, ы; e elsewhere.
When written as ё in Russian, transliterate as yě or ě.

RUSSIAN AND ENGLISH TRIGONOMETRIC FUNCTIONS

Russian	English	Russian	English	Russian	English
sin	sin	sh	sinh	arc sh	sinh ⁻¹
cos	cos	ch	cosh	arc ch	cosh ⁻¹
tg	tan	th	tanh	arc th	tanh ⁻¹
ctg	cot	cth	coth	arc cth	coth ⁻¹
sec	sec	sch	sech	arc sch	sech ⁻¹
cosec	csc	csch	csch	arc csch	csch ⁻¹

Russian	English
rot	curl
lg	log

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CASES OF LABORATORY CONTAMINATION BY BOLIVIAN HEMORRHAGIC FEVER.

Ye. V. Leshchinskaya, M. P. Chumakov, I. N. Martynenko, Ye. A. Tkachenko, L. B. El'bert.

Institute of poliomyelitis and virus encephalitides of AMN of the USSR.

Bolivian hemorrhagic fever is endemic for South America and to Soviet researchers it is known only according to published data. In connection with this is of interest the description of 3 cases of this disease, which arose as the result of interlaboratory contamination among the coworkers of the of AMN of the USSR.

The clinical picture of Bolivian hemorrhagic fever (BGL) is described in the series/number of communications/reports (R. Mackenzie et al, 1963, 1964; A. Shelokov, 1964). Among them attracts attention work Stinebaugh et al (1966), which give the detailed information

about 4 cases BGL, which arose among scientific workers and service personnel of the viruscological laboratory, expanded/scanned by American scientists in Bolivia with purpose of the study of etiology BGL. In these cases it was possible to assume the interlaboratory character/nature of contamination, although the prolonged stay sickened in endemic focus BGL does not make it possible to judge about this in categorical form. The present communication/report is devoted to the description of the cases of diseases, which arose in laboratory workers, who are located out of endemic focus. The results of clinical observation and inspection/examination of these patients are of definite interest for Soviet researchers, familiar with BGL only on literature.

In 1972 in the laboratory of the hemorrhagic fevers of the institute of poliomyelitis and virus encephalitides of AMN of the USSR were conducted the investigations, bonded with the study of virus Machupo - liberation/excretion of virus from the organs/controls of rodents, delivered from Bolivia, the work with the infected animals and the culture of tissue, the preparation of antigens. Of 6 people, who participated in these investigations, 3 scientific workers and 2 laboratory assistants sickened by BGL. Diseases appeared for a period of 2.5 months with 3rd and 6- weekly intervals. The absence of indications of any emergency situations and injuries makes it possible to assume the respiratory way of

contamination. It is most probable that the contamination occurred with work with the infected animals or during the preparation/manufacture of the suspension of virus.

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Clinically in two cases disease flowed/occurred/lasted with average/mean severity and in one - it is heavy. Feverish period lasted 7-8 days, temperature was characterized by spreads/scopes in 1.5-2°. All patients complained about first, muscular and joint pains, repeated chills, vomiting, absence of appetite. Against this general/common/total toxic background from 4-5 days of disease/sickness/illness/malady appeared the syndromes, characteristic for this disease:

1. Progressive decrease in arterial pressure to critical numerals - 80/40-90/50. In one of the patients during the attempt to sit down in bed developed short-time hemodynamic shock.

2. Hemorrhagic syndrome which was expressed by petechial rash in axillary regions (along posterior axillary line) in all patients and in one patient with light uterine and nasal hemorrhage.

3. Damage/defeat by mucosa of mouth and with development of

surface small ulcers, covered with white fibrinogenous exudation. The same exudation appeared at tonsils. Mucosa of sky, cheeks and gums was hyperemized, edematic, turbid. Patients complained about dryness in mouth, pain in throat with ingestion, a feeling of rawness.

4. Increase and sickness with palpation of lymph nodes, mainly of submaxillary and neck, pastiness surrounding soft tissues. Latter/last two syndromes - aphthous damage/defeat mucosa of mouth and poly-adenitis - were observed in 2 patients. The tremor of hands, jaws and tongue, disturbances/breakdowns of statics had not one of our patients. The manifestations of encephalopathy occurred in one patient and consisted in sharp irritability, precipitation of memory, increased somnolency.

Changes in the peripheral blood were very sharp and they were characterized, first of all, by an incidence/drop in the total number of leukocytes to 2000-2500 and by an increase in the quantity of young forms. Besides leucopenia, it was typical so thrombocytopenia with lowering in the quantity of thrombocytes to 60 thousand. Just as with other types of hemorrhagic fevers, at the 2nd week of disease/sickness/illness/malady appeared atypical monocytoid cells - virocytes. In the urine of patients was determined the protein: albuminuria reached 100/00, sediment in this case was sufficiently skimpy - 5-8 erythrocytes, kidney epitheliums, unitary transparent

cylinders. The specific gravity/weight of urine remained normal, the signs of acute/sharp kidney dericiency was not. At the same time pains into the region of loin and the positive symptom of Pasternatskiy were in all patients. From other investigations is of interest the coiling system of the blood. In 2 patients were noted the signs of hypocoagulation in the form of the elongation of thrombin time, in one case this was matched with a reduction in the tolerance of plasma to heparin, in other - with a significant increase in the quantity of free heparin. In patients with the heavy course of the disease (see extraction from the history of disease/sickness/illness/malady of 30 years) during the examination of the functional state of the liver was revealed an increase in the enzymes - asparataminotransferase and alaninaminotransferase.

The treatment of patients was conducted by symptomatic substances. Furthermore, into the scheme of treatment was included prednisolone. All patients recovered. The period of convalescence was characterized by the prolonged asthenia, intensified by appetite and noticeable addition in weight, shedding of hair, almost to full/total/complete baldness with their subsequent reduction during half a year.

In 2 patients during virusological investigation from the blood was isolated the virus BGL. The results of serological inspection/examination are represented in Table 1.

In conclusion we bring as the illustrations of extraction of 2 histories of disease/sickness/illness/malady.

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Table 1. Results of the serological inspection/examination sick BGL.

(1) Вид реакции	(2) Антигены	(3) Титры антител																	
		(4) Больная Ш.						(5) Больная А.						(6) Больная Т.					
		1д	12д	15д	3 мес.	6 мес.	9 мес.	1д	4д	5д	10д	30д	5 мес.	8 мес.	1д	16д	1,5 мес.	4 мес.	7 мес.
РСК (7)	Мачупо	0	0	1:8	1:8	0	0	0	0	0	0	1:32	1:16	0	0	1:4	1:32	1:16	0
	КГЛ	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
РДГА (7)	Мачупо	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	+	+	+
	КГЛ	1:4	1:4	+	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0
РН (7)	Мачупо	0	0	1:8	1:8	1:4	1:2	0	0	0	0	1:32	1:16	1:8	0	0	1:64	1:32	1:8

Note: + serum reacts only in the undiluted form/species.

Key: (1). Form/species of reaction. (2). Antigens. (3). Titers of antibodies. (4). Sick Sh. (5). Sick A. (6). Sick T. (7). Machupo.

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Sick T. of 30 years. It sickened sharply on night from 4/III on 5/III-1972 g., when it appeared a chill, pains in joints, muscular pains, temperature of 37.5°, 5/III state remained the same, temperature of 37.5°, 6/III - temperature of 39° with periodic

lowering for several hours to 37°. New increases in the temperature were accompanied by the staggering chills (Fig. 1). To previous complaints was added the dryness in mouth and a feeling of rawness in throat.

7/III it is hospitalized. After entry the state of average/mean severity, complaint of overall weakness, rheumatic pain in muscles and joints, chills, dryness in mouth, small pain with ingestion. With inspection was noted hyperemia and graining of the posterior wall of the pharynx and the soft palate. Lymph nodes did not palpate, skin pure/clean. Arterial pressure by 120/70. Relative bradycardia - pulse 76 into 1' at temperature of 37.8°. The tones of heart are muted, systolic noise on head (in anamnesis rheumatism). In the lungs it is pure. Tongue is lined, moist. Stomach soft, painless, small sensitivity with palpation in the region of the liver. Neurologic status without deflections from norm.

8/III state remained average/mean severity. Night it slept badly/poorly due to the chills, alternating with perspirations. Was strengthened pain in throat. There was one time abundant vomiting. Face and scleras are slightly hyperemized. Hyperemia of pharynx became sharper and was disseminated to the hard palate. Mucosa of mouth is pure/clean. Appeared an increase and light sickness with the palpation of submaxillary lymph nodes to the left. On skin, along

posterior axillary lines appeared abundant petechiae. Arterial pressure by 110/60, is held relative bradycardia. On organs/controls there are no new data.

9/III the health of patient somewhat was improved against the background of lowering the temperature.

The breakage T. of 30 years.

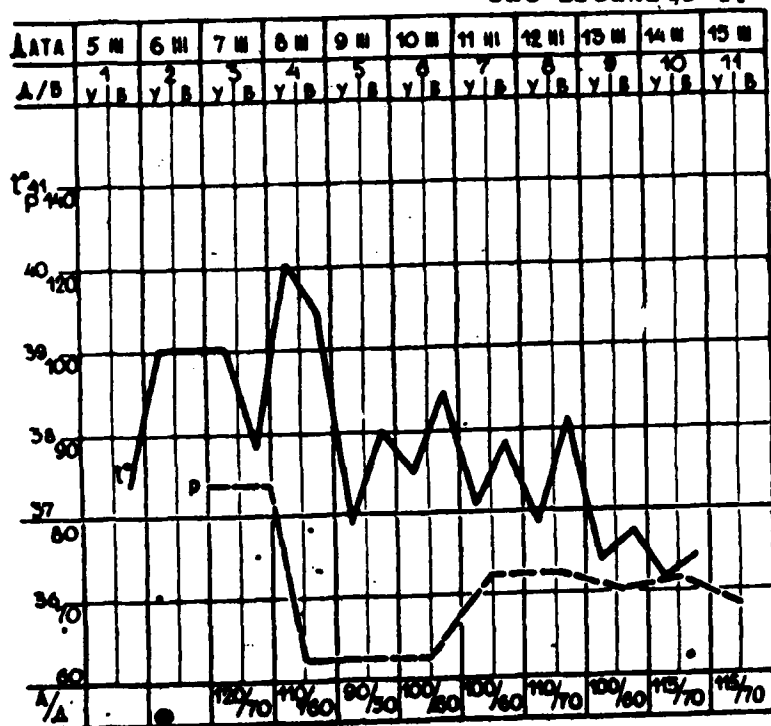


Fig. 1.

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However, 10/III anew began to grow on intoxication, even more were strengthened pains in throat, dryness in mouth, anew appeared multiple chills, were muscular joint pains, pains in spin. Twice howled vomiting. Mucosa of pharynx, soft and hard palate is sharply hyperemized, edematic, grained, on right tonsil the dense grayish-white exudation, which is removed/taken with difficulty.

Mucosa of mouth lackluster, is edematous, with the sections, covered with whitish exudation. The neck was pasty palpate morbid sensitive submaxillary lymph nodes. Arterial pressure by 100/60. There are no hemorrhagic manifestations, besides the described above hemorrhagic rash. The tones of heart became more mute, the left boundary of heart was expanded to the left on 0.5 cm from mamillary line. In the lungs harsh respiration, to the right under scapula moist bubbling rales, is many scattered dry wheezes. Dry cough. Tongue is thickened, lined. Stomach soft, painless, liver and spleen do not palpate, upper boundary of the liver on IV edge/fin. There are no meningeal signs. Is irritable, negative and at the same time somnolent. Light tremor of hands. In other respects neurologic status without deflections from norm. By day in patient, during the attempt to get up from bed, appeared sharp pallor, the cyanosis of mucosas, pulse became soft, 60 shocks per minute. Arterial pressure by 100/60-90/60, after 10-15 minutes state was improved.

During the subsequent 3 days (11/III, 12/III, 13/III) the state of patient remained previous. Appeared fresh aphthae on mucosa of cheeks, increased neck lymph nodes. From the side of internal organs/controls the data were the same, decreased only a quantity of moist wheezes in the lungs.

From 15/III state became satisfactory of change in the pharynx

began to undergo reverse development, blood pressure was normalized. Remained only sharp overall weakness, increased irritability, the periods entirely correct alignment in time and precipitation of memory to the nearest events. The tremor of hands and tongue it was not noted 23/III, in connection with the absence of any pathological changes from the side of internal organs/controls and by the standardization of picture in the pharynx of patient it was discharged home.

The analyses of the blood.

	7/III	9/III	10/III	11/III	13/III	15/III	17/III	27/III
(1) Эритроциты (млн.)	1,5	4,45	4,45	4,4	—	—	4,45	—
(2) Гемоглобин	15,2	14	15,2	16,6	14,8	15	11,8	14,8
(3) Лейкоциты	2600	2000	2700	2000	23,00	4600	7600	8600
(4) Эозинофилы	1	—	—	—	—	—	—	—
(5) Юные	—	—	3	1	—	—	—	—
(6) Палочкоядерные	24	24	28	53	11	4	6	5
(7) Сегментоядерные	53	52	40	30	33	30	50	67
(8) Лимфоциты	12	11	12	12	33	41	32	15
(9) Моноциты	7	5	2	4	11	12	10	18
(10) Ретикуляр. кл.	3	3	5	—	2	—	—	—
(11) Атипичные мононуклеары	—	—	3	—	7	12	—	—
(12) Плазматич. кл.	—	1	7	—	3	1	2	—
POЭ	10	10	6	10	10	20	26	19
(13) Тромбоциты (тыс.)	270	176	140	66	—	—	374	302

Key: (1). Erythrocytes (million). (2). Hemoglobin. (3). Leukocytes.
 (4). Eosinophils. (5). Young. (6). Stabnuclear. (7). Segmentonuclear.
 (8). Lymphocytes. (9). Monocytes. (10). reticular cell. (11).
 Atypical mononuclears. (12). Plasmatic cell. (13). Thrombocytes
 (thousand).

Analyses of urine.

	9/III	10/III	11/III	13/III	15/III	16/III	18/III	20/III	22/III	23/III
(1) Удельный вес	1016	1020	1028	1020	1026	1025	1030	1030	1028	1030
(2) Белок в ‰	0,033	0,165	1,32	6,6	9,9	8,9	0,66	—	—	—
(3) Сахар	—	—	—	+	+	+	+	+	+	+
(4) Лейкоциты	1-3	1-6	8-12	6-7	3-5	3-5	2-3	0-1	1-2	0-1
(5) Эритроциты	5-7	3-5	7-10	с.с.	1-2	1-3	с.с.	1-2	1-2	1-2
(6) Цилиндры	—	—	—	—	—	—	—	—	—	—
(7) Глистные	с.с.	с.с.	0-1	с.с.	с.с.	с.с.	—	—	—	—

Key: (1) specific gravity/weight. (2). Protein in o/oo. (3). Sugar.
 (4). Leukocytes. (5). Erythrocytes. (6). Cylinders. (7). transparent.

Coiling system of the blood 11/III-72 c.

(1) Толерантность плазмы к гепарину в минутах	28	(3) норма 11—16
(2) время Квика в сек.	25	(3) норма 25
(4) Тромбиновое время в сек.	40	(3) норма 15
(5) фибриноген «В» в мг	4	
(6) фибринолитическая активность в минутах	240	(3) норма 150—200

(7) Сахар в крови 20/III — 232 ⁽⁸⁾ мг % 23/III — 123 ⁽⁸⁾ мг %	(9) Электрокардиограмма от 9/III — синусовая брадикардия. Изменения миокарда левого желудочка
(10) белок общий в сыворотке крови 15/III — 6.48	
(11) белковые фракции сыворотки крови 15/III	(12) Электрокардиограмма от 13/III — изменения миокарда левого желудочка

(13) Альбумины	(14) альфа ₂	(15) альфа ₁	(16) бета	(17) гамма	(18) гамма
63.3	5.1	7.1	9.2	15.3	15.3

Key: (1). Tolerance of plasma to heparin in minutes. (2). Time of Quick in s. (3). norm. (4). Thrombin time in s. (5). Fibrinogen of "B" in mg. (6). Fibrinolytic activity in minutes. (7). Sugar in blood. (8). mg. (9). Electrocardiogram from 9/III - sinus bradycardia. Changes in the myocardium of left ventricle. (10). Protein general/common/total in blood serum 15/III - 6.48. (11). Protein fractions of blood serum 15/III. (12). Electrocardiogram from 13/III - change in myocardium of left ventricle. (13). Albumins. (14). Alpha. (15). beta. (16). gamma.

Results of the examination of the function of the liver.

	9/III	13/III	22/IV
(1) Холестерин в %	150	120	180
(2) Билирубин общий в %	0,5	0,2	0,3
(3) Билирубин не прямой	0,5	0,2	0,3
(4) Сулемовая проба	2,8	—	0,2
(5) Альдолаза	—	6	—
(6) Аспаратаминотрансфераза	66	207	64
(7) Аланинаминотрансфераза	60	157	62
(8) Коэффициент	1,1	1,3	1,1
(9) Тимоловая проба	2	2	2

Key: (1). Cholesterol in. (2). Bilirubin (general/common/total in.)
 (3). Bilirubin of indirect. (4). sublimate test/sample. (5).
 Aldolase. (6). *aspartate aminotransferase*. (7). *alaninaminotransferase*.
 (8). Coefficient. (9). Thymol test/sample.

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Prothrombin index from 17/1 - 1040/o.

Functional tests/samples of the liver from 13/1 - without deflections from norm.

The treatment conducted. Prednisolone it is intramuscular from 3 days of disease/sickness/illness/malady to 16 days of disease/sickness/illness/malady. Total quantity of preparation to course 205 mg.

Intravenous pouring in of polyglucine, neocompensane, ascorbic acid, cocarboxylase.

Sick Sh. of 35 years per 31/XII-71 g. to 8/I-72 g. felt the general/common/total malaise, weakness, there were small catarrhal phenomena from the side of the upper respiratory tract. Temperature was normal, it continued to work. 9/I state deteriorated, temperature of 38.5°, chill, headache 10/I, temperature of 37.3-38.5°, pain in spin, muscles and joints, headache, chill. 11/I temperature of 37.5-38.5° (Fig. 2), single vomiting, sanious liberations/excretions from nose, blood-containing from vagina. 12/I patient is hospitalized. State after entry sufficiently heavy. Complains about strong headache, chill, pains in the eyeballs, pain in loin and in epigastral region, dryness in mouth. There is no rash on skin, bruises in the places of injections. Expressed scleritis and conjunctivitis. Mucosa of mouth the clean, pale, posterior wall of the pharynx is hyperemized, grained. Lymph nodes are not increased. Bradycardia, arterial pressure by 90/60. The tones of heart are muted. Tongue is lined, dry, stomach soft, morbid with palpation in epigastral region. The liver palpates on 2 cm, is morbid, spleen increased. Symptom of Pasternatskiy sharply positive from both sides. Nervous system without pathology. Patient is flaccid, somnolent.

During the subsequent two days the state of patient was medium-weight; chills were interrupted, it was noted small sweating, sharp weakness, apathy. From 15/I state somewhat was improved, temperature was normalized, however, remained overall weakness, bradycardia (pools 60 ud, hypotonia 85-90/60). In this same period somewhat was strengthened the dullness of heart tones with the state of preservation of the normal boundaries of heart. The symptom of Pasternatskiy became negative.

The patient Sh. of 35 years.

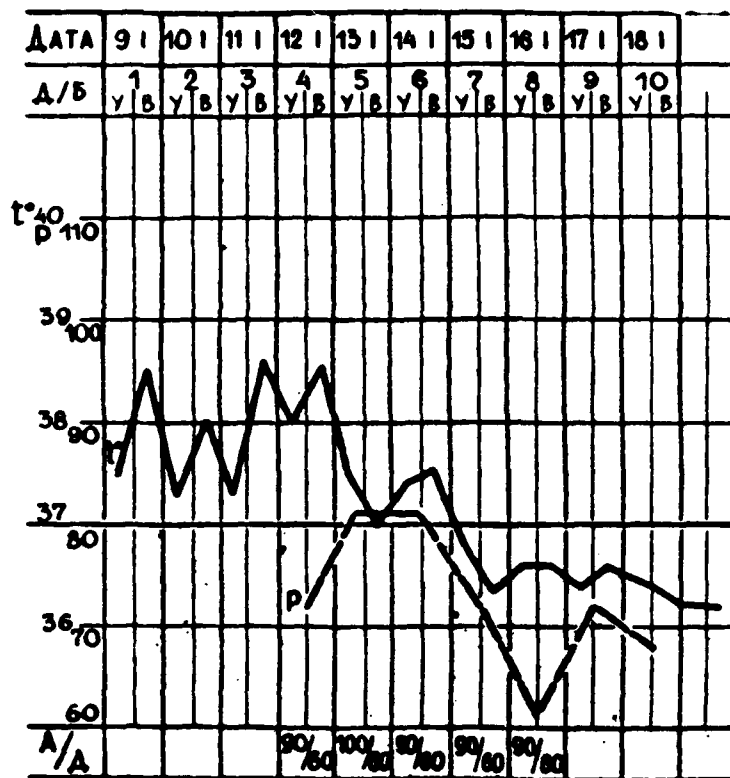


Fig. No 2.

Analyses of the blood.

	12/I	15/I	19/I	25/I
1. Эритроциты млн.	43	41		—
2. Гемоглобин	14,4	12,6		12,4
3. Лейкоциты	2400	3000		5400
4. Эозинофилы	—	2		—
5. Юные	1	—		—
6. Палочкоядерные	17	5		2
7. Сегментоядерные	34	34		48
8. Лимфоциты	26	41		43
9. Моноциты	15	9		7
10. Атипичные мононуклеары	6	10		
11. Плазматические клетки	1	1		
12. РОЭ	12	12		15
13. Тромбоциты (тыс.)	172 (157)	—	287 (5)	
14. Время кровотечения по Дукке	2 м 30 сек.		1 м 30 сек.	

Key: (1). Erythrocytes of million. (2). Hemoglobin. (3). Leukocytes. (4). Eosinophils. (5). Young. (6). Stabnuclear. (7). Segmentonuclear. (8). Lymphocytes. (9). Monocytes. (10). Atypical mononuclears. (11). Plasma cells. (12). ROE. (13). Thrombocytes (thousand). (14). Time of hemorrhage according to Duke. (15). s.

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Sickliness in epigastral region decreased. From 25/I (17 day of disease/sickness/illness/malady) blood) blood pressure was stabilized at the level 110/70-100/60, all pathological symptoms disappeared. Remained only overall weakness, enervation. 26/I patient was discharged home.

General/common/total protein of blood serum 14/I - 7.87.

Protein fractions:

(1) альбумины	(2) альфа ₁	(3) альфа ₂	(4) бета	гамма
51,1	4,4	9,5	14,6	20,4

Key: (1). albumins. (2). alpha. (3). beta. (4). gamma.

Electrocardiograms from 17/I and 24/I - change in the myocardium of left ventricle.

Diastasis of urine from 19/I - 8 unity.

The treatment: prednisolone it is intramuscular from 12/I (4 day of disease/sickness/illness/malady) to 26/I (18 day of disease/sickness/illness/malady). Total dose to course 300 mg.

Intravenous introduction of nemodese, plasma, polyglucine, cocarboxylase, ascorbic acid.

The described cases of the disease BGL doubtless are bonded with the interlaboratory contamination which occurred most likely respiratorily. The interest of these cases consists so in the fact that they demonstrate the diverse variants of the clinical course BGL, which differ from each other by severity, degree of damage/defeat by mucosa of mouth and, lymph nodes. In the absence of aphthous stomatitis and poly-adenitis clinical differential diagnosis with other types of hemorrhagic fevers, especially Crimean

hemorrhagic fever, considerably milder, because of the similarity of clinic, hematologic shifts and the character/nature of the damage/defeat of vascular system. Attention is drawn to changes in the functional state of the liver (increase in the enzymes) in one of our patients (sick g. of 30 years), which indicates its interest in pathological process. It is known that despite all types of hemorrhagic fevers (Crimean, Marburg, hemorrhagic fever with kidney syndrome) in many instances with lethal outcome is revealed necrotic hepatitis (I. I. Gets, 1965; Ye. V. Leshchinskaya, 1976). C Child with co-authors (1976) described the foci of necrosis in hepatic parenchyma in the 2nd patients, who was killed from BGL. The reason for these changes in the liver up to now remains unexplained. In the literature is discussed the possible role of hypoxia, which appears as a result of sharp circulatory disturbances/breakdowns. Is not excluded also the straight/direct action of virus on hepatic parenchyma.

The analyses of urine.

	13/1	15/1	25/1
1. Уд. вес	1021	1020	1017
2. Белок %	0,165	следы (2a)	нет (2b)
3. Лейкоциты	1-2	ед. (3a)	3-5
4. Эритроциты свежие	16-18	2/3	нет (2b)

Key: (1). Ud weight. (2). Protein. (2a). tracks. (2b). no. (3). Leukocytes. (3a). unit. (4). Erythrocytes (fresh).

Conclusions/derivations.

1. Were observed 3 cases BGL, that arose as a result of interlaboratory contamination.
2. Most probable way of contamination was respiratory way.
3. Clinical symptomatology was typical for this disease with certain variability in each individual case.

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REFERENCES

- Гед И. И. (1965) — Материалы к изучению патологической анатомии так называемой тульской геморрагической лихорадки. Труды Института полиомиелита и вирусных энцефалитов АМН СССР. т. VII, 129.
- Лещинская Е. В. (1967) — клиника крымской геморрагической лихорадки и сравнение ее с геморрагическими лихорадками других типов. Автореф. дисс. докт., М.
- Child C., Mackenzie R., Valverde L., Johnson K., 1967 — Bolivian hemorrhagic fever Aron Rath. v. 83, 434.
- Mackenzie R., Valverde L., Garron A., Beye H. (1963) — Fiebre hemorrhagica en Bolivia. Rev. Rupl. Boliviana, 19, 11.
- Mackenzie R., Beye H., Valverde L., Garron H. (1964) — Epidemic hemorrhagic fever in Bolivia. Amer. J. Trop. Med. Hyg., 13, 620.
- Shelokov A., (1964) — Hemorrhagic fevers in the American symposium on some aspects of hemorrhagic fevers in the Americas New York.
- Stinebaugh B., Schoeder F., Johnson K., Mackenzie R., Entmistle G. (1966) — de Alba T., and Bolivian hemorrhagic fever the Amer. J. of Med., 40, 217.

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INSOLATION AND IDENTIFICATION OF NEW STRAINS OF THE VIRUS OF BOLIVIAN
HEMORRHAGIC FEVER FROM THE ORGANS OF RODENTS CALOMYS CALLOSUS AND
FROM THE BLOOD OF MAN.

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Summary.

Upon the virusological inspection/examination of the
organs/controls of rodents, caught in the focus of Bolivian
hemorrhagic fever in Bolivia, is isolated 4 strains: C-80/81, C-58,
C-79, C-84. The liberation of viruses proved to be possible only from
the organs/controls of rodents Calomys Callosus. Furthermore, from
the blood sick BGL in acute/sharp feverish period is
isolated/insulated strain T-1610. All strains are identified as the

strains of virus Machupo.

Introduction.

The causative agent of Bolivian hemorrhagic fever (BGL) - virus of Machupo was for the first time isolated/insulated during May 1963 (prototype strain "Karvallo") from the spleen of the killed person (M. K. Johnson et al, 1965). Reservoir and carrier of virus Machupo in nature proved to be rodents *Calomys callosus* that by the confirmed repeated liberations of virus from their organs/controls (K. M. Johnson et al, 1966).

Because of the specific related interrelations of the complement-binding antigens the viruses Machupo, Takaribe, Hunin, Ampari, Latino, Tamiami, Parana and Pinchunde were joined into antigenic subgroup Takaribe. The differentiation of viruses in this subgroup is possible with the aid of neutralization reaction.

With electron-microscopic examination is established/installed the morphological similarity of the viruses of complex Takaribe to the virus of lymphocytic choriomeningitis and the virus of Lass isolated in 1969 in Nigeria (S. M. Buckley, I. Casals, 1970).

On this basis, and also on the basis of the data about the crossed antigenic bonds, revealed/detected with the aid of the method of immunofluorescence, is proposed to secrete these viruses into the new taxonomic group arena viruses with the subgroup of viruses of Takaribe, considering the prototype of group the virus of lymphocytic choriomeningitis (E. A. Murphy et al, 1969); W. P. Rowe et al, 1970; R. W. Speir et al, 1970; W. P. Rowe et al, 1970).

In the present report we have represented results on the identification of the strains, isolated from the organs/controls of rodents *Calomys callosus* and from the blood of patient in the case of disease in laboratory coworker, who conducted the virusological inspection/examination of the organs/controls of rodents, caught in Bolivia.

Materials and methods.

1. Inspection/examination of organs/controls of rodents. In 1971 in the focus of the diseases of BCL in Magdalen city and its vicinities of Beni's department province of Itenes in Bolivia were caught by animal traps the mouse-like rodents of different types (T.

P. Povalishina, 1972). Organs from each rodent (liver, kidneys, spleen) were delivered into the Soviet Union in the form/species frozen with the aid of liquid nitrogen.

The virusological inspection/examination of the organs/controls of rodents was conducted employing the conventional procedure, using for contamination 1-2 diurnal neonatal white mice (NBM). In all it is inspected of 22 tests/samples from 25 rodents: 14 - *Calomys callosus*, 9 - *Zygodontomys*, 1 - *Mus musculus* and 1 - *Oryzo-* (in three tests/samples organs/controls are joined from the 2nd rodents of one form/species). For *zarazheniya* contaminating NBM they took on 0.01 ml by 10o/o of suspension of the mixture of organs/controls (liver, kidney, spleen), prepared in physiological solution. 10o/o suspension of brain in the physiological solution of those sickened or been killed after 4-th for NBM used for a subinoculation and simultaneously used it as antigen in RSK with the hyperimmune serum, prepared against prototype strain (Karvallo) of virus of Machupo.

2. Inspection/examination of people's blood in cases of disease of BGL of laboratory workers. In 1972 during the investigations, bonded with the study of virus of Machupo (liberation of virus from the organs/controls of rodents, preparation of antigens, work with the infected animals and a culture of tissue), 1 scientific worker even 2 laboratory assistants sickened by Bolivian hemorrhagic fever

(Ye. V. Leshchinskaya with co-auth., 1972).

The blood from 3 patients in acute/sharp feverish period they inspected virusologically with the aid of the intra-cerebric contamination of the neonatal mice (NBM). For the inoculation of NBM usually used the mixture of erythrocytes and sera (1:1) and breeding/culture/dilution of blood 1:10 in physiological solution. The brain of the suspicious and fallen mice (100/o suspension) was used for a successive subinoculation and simultaneously was checked in RSK as antigen with antiserum of Machupo. To these coworkers' disease/sickness/illness/malady in laboratory was not conducted the work with the prototype strains of viruses of Machupo, Hunin, Takaribe or with other representatives of group of Takaribe.

3. Strains of viruses, used for identification. Virus of Machupo (prototype strain Karvallo and strain CTV 4517), virus of Takaribe (strain TR 11573) and antigen of the virus of Hunin (strain HJ 15950 and CTV 4452) were obtained in 1966 in the lyophilized form/species from center of VOZ by arbo viruses through doctor Kazal's. After 5-6 subinoculations to NBM the viruses were used in the form 100/o of cerebriic suspension, purified by centrifuging with 3-4000 r/min during 20 minutes.

4. RSK. Antigen for RSK was prepared from cerebriic tissue of

sick NBM with the aid of borate-salt of extraction either saccharose-acetone or chloroform obrabotki. A setting RSK realized on a microtitrator "Takachi" employing the conventional procedure, using 2 doses of complement. Sera to setting RSK heated 30 minutes with 58°C.

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5. RN. Neutralization reaction conducted through method suppressions of virus interference against virus of vesicular stomatitis (VVS) in culture of tissue of cells of chicken/gallinaceous embryos. Were used 2 modifications: either testing the mixtures of 10 multiple breedings/cultures/dilutions of virus and one concentration of serum (1:2), or testings of the mixtures of the growing on double breedings/cultures/dilutions of serum and one concentration of the investigated virus (100 doses). The mixture of virus with serum before introduction into cultures preliminarily withstood/maintained 1.5-2 hours at room temperature. For the titer of antibodies they accepted the great breeding/culture/dilution of immune sera which prevented the development of the interfering effect/action of virus in the half of culture tubes KKE.

6. Sera. Specific hyperimmune sera obtained by the immunization

of guinea pigs, white rats and rabbits, and ascitic fluids/liquids with the aid of the immunization of adult white mice.

7. RDPA. For setting of reacting diffusion precipitation in gel of agar (RDPA) was used micromethod on microscope slides. Antigens for RDPA were prepared with the aid of the concentration of cerebriic borate-salt antigens, and also virus-containing liquid culture medium KPZM, by polyethylene glycol with a molecular weight of 2000 and 6000. In detail the procedure of the concentration of the antigens of viruses by polyethylene glycol is described by M. P. Chumakov with co-authors (1968).

Results.

Of 22 inspected into NBM tests/samples of the organs/controls of the rodents 4 forms/species it is isolated of 4 strains: C-58, C-79, C-80/81, C-84. All 4 strains were isolated/insulated of the organs/controls only of the one form/species of rodents - *Calomys callosus*. Furthermore, was isolated/insulated virus - strain T-1610 - from the blood of the sickened coworker, who accepted participation in the investigations, bonded with the liberation of virus from the organs/controls of Bolivian rodents.

The disease of NBM after primary contamination they observed to

9-16 days from the moment/torque of the infection of animals. Through 2-3 cerebriic subinoculations to NBM incubation period was shortened to 7-9 days and subsequently, in proportion to subculturing, it remained in effect permanent. The symptoms of disease/sickness/illness/malady in the neonatal white mice were noted from 7 days after contamination and were developed in the form of the delay of growth, apathy, disturbance/breakdown of motor coordination. After turning of animal for tail appear the tonic and clonic spasms, the atoxic state, which is accompanied by times by rigidity. Lethality is observed not in all infected animals. Some animal with explicit clinical manifestations diseases/sicknesses/illnesses/maladies to 18-21 days from the moment/torque of contamination survived. The specificity of the clinical manifestations of disease/sickness/illness/malady in NBM was confirmed by regular development/detection in the brain of the sickened little mousera of the complement-binding antigen and by successful subinoculations of virus on other groups of NBM.

In RSK the antigens of strains C-80/81, C-79, C-84, C-58 and T-1610 positively reacted both with the antiseraums to these five strains and with antiseraums to the prototype strains of viruses Machupo, Hunin and Takaribe (Table 1). In turn, the antigens of viruses of Machupo, Hunin and Takaribe positively reacted with antiseraums to strains C-80/81, C-58, C-79, C-84 and T-1610.

These results are confirmed also in the experiment/experience of the checked titration of antigens and sera in RSK (Table 2). The newly isolated strains on data of RSK proved to be identical between themselves and to the prototype strain (CTV 457) of virus of Machupo; they have the related bond of antigens with other terms of the complex of viruses of Takaribe.

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Table 1. Identification in HSK of the strains, isolated from rodents and from the blood of man.

(1) Antigeny (4-SAE)	(2) Титры сыворотки					(4) Мачупо (CTV 4517)	(5) Хуни (J-798)	(6) Такарибе (TR 11673)	(7) КГЛ (Патн.)	(9) Норм. с-ва мса.
	C-80/81	C-79	C-88	C-84	T-40 ¹					
C-80/81	64	32	32	16	32	64	32	16	0	0
C-79	64	32	32	16		64	32	16	0	0
C-88	64	32	64	16		64	32	16	0	0
C-84	64	32	64	16		64	16	16	0	0
T-1610	64				32	64	16	16	0	0
(*) Мачупо (CTV 4517)	64	32	32	16	32	64	32	16	0	0
(*) Хуни (HJ 16960)	16	16	16	8	8	32	64	16	0	0
(*) Такарибе (TR 11673)	16	8	16	4	4	16	32	32	0	0
(*) КГЛ (Патн.)	0	0	0	0	0	0	0	0	128	0
(*) Норм. антиген	0	0	0	0	0	0	0	0	0	0

Key: (1). Sera. (2). Titers of sera. (3). Antigens. (4). Machupo.
 (5). Hunin. (6). Takaribe. (7). KGL (Pyath.). (8). Normal serum,
 guinea pigs. (9). KGL (Pyathits.). (10). N. antigen.

FOOTNOTE 1. T-40 - serum of reconvalescent T, undertaken on 40 days
 after the beginning of disease.

2. Reciprocal value of the greatest breeding/culture/dilution of
 serum, which gave positive reaction. ENDFOOTNOTE.

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In the reaction of diffusion precipitation in agar (RDPA), applied by us for the first time for studying the viruses of group Takaribe, was possible to conduct serological differentiation between viruses of Machupo and relationship of strain C-80/81 with the prototype strain of virus Machupo.

As can be seen from Table 3, the serum, prepared to virus Takaribe, caused the formation of the strip of precipitation with antigen Takaribe in breeding/culture/dilution of 1:16, while with antigen C-80/81 and Machupo reacted only in breeding/culture/dilution of 1:2. Antiserums against viruses C-80/81 and Machupo only in the undiluted form/species weakly reacted with antigen of Takaribe, giving positive reaction with homologous antigens in breeding/culture/dilution of 1:16.

With the aid of neutralization reaction (Table 4), is carried out the final identification of the newly isolated strains.

As can be seen from Table 4, strains C-80/81, C-79, C-58, C-84 and T-1610 according to the data of neutralization reaction, they

have intimate immunological bond both between themselves and with prototype strain Karvalio or virus Machupo and at the same time they easily are differentiated from virus of Takaribe. The results of the experiment/experiences of neutralization make it possible to consider the rodents *Calomys callosus* isolated from organs/controls and from the blood of sick person viruses the strains of virus Machupo.

The inspection/examination of the blood serum of the endured person in dynamics with the antigens of the viruses of group of Takaribe and the antigen of virus KGL (Table 5), came to light/detected/exposed the diagnostic build-up/growth of antibodies in RSK with antigens T-1610, C-80/81 and Machupo (CTV 4517) from zero to 1:32 after expiration of month since the beginning of the disease. The titers of sera in RSK with the antigens of Hunin and Takaribe were 4 and 8 times, correspondingly, lower than with antigen of Machupo. This, by the way, indicates the possibility of the serological differentiation between the causative agents of Argentinean (virus of Hunin) and Bolivian (virus of Machupo) of hemorrhagic fevers.

Table 2. Identification in the reaction of the joining of the complement of strain C-80/81 with viruses of Machupo and Takaribe (checkered titration).

(5) Антигены	(1) Сыворотки			
	C-80/81	(2) Мачупо (CTV 4517)	(3) Такарибе (TR 11573)	(4) Норм. с-ка м. св.
C-80/81	64/128 ⁽⁶⁾	64/128	32/32	0
(2) Мачупо (CTV 4517)	64/128	64/128	32/32	0
(3) Такарибе (TR 11573)	16/32	16/64	32/128	0
(4) Норм. антиген	0	0	0	0

Key: (1). Sera (2). Machupo. (3). Takaribe. (4). N. serum, guinea pigs. (5). Antigens. (6). N. antigen.

FOOTNOTE 1. In numerator - the titer of antigen, in denominator - the titer of serum. ENDFOOTNOTE.

Table 3. Identification in the reaction of diffusion precipitation in agar of strain C-80/81 with viruses Machupo and Takaribe (checkered titration).

(5) Антигены	(1) Сыворотки			
	C-80/81	(2) Мачупо (CTV 4517)	(3) Такарибе (TR 11573)	(4) Норм. с-ка м. св.
C-80/81	8/16	8/16	+/2	0
(2) Мачупо (CTV 4517)	8/16	8/16	+/2	0
(3) Такарибе (TR 11573)	2/+ ⁽⁶⁾	2/+	8/16	0
(4) Норм. антиген	0	0	0	0

Key: (1). Sera. (2). Machupo. (3). Takaribe. (4). N. serum, guinea pigs. (5). Antigens. (6). N. antigen.

FOOTNOTE 1. + serum or antigen reacts in RDPA only in the undiluted form/species. ENDFOOTNOTE.

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Table 4. Neutralization reaction according to the method of interference. Identification of the strains, isolated from rodents and from the blood of man.

(1) Сыворотки		(2) Титры и индексы нейтрализации сыворотки					
(3) Вирусы	C-80/81	C-79	C-58	C-84	T-40	(4) Мачупо (Карвалло)	(5) Такарибе (TR 11573)
C-80/81	7.0/3.0 ¹	7.0/3.0	5.0/2.7	5.0/2.3	6.0/NI	6.0/3.0	3.0/1.6
C-79	7.0/3.2	7.0/3.0	5.0/2.3	5.0/2.1		7.0/3.6	3.0/1.0
C-58	7.0/3.6	7.0/2.7	5.0/2.6	5.0/2.3		6.0/3.1	3.0/1.7
C-84	6.0/3.2	6.0/3.2	5.0/2.3	5.0/2.6		6.0/3.0	3.0/1.9
T-1610	6.0/				6.0/NI	6.0/NI	3.0/NI
④ Мачупо (Карвалло)	6.0/2.6	7.0/3.6	5.0/2.6	5.0/2.3	6.0/NI	6.0/3.6	3.0/1.6
⑤ Такарибе (TR 11573)	0/0.7	0/0	0/0.3	0/0	0/NI	0/0.3	8.0/4.8

Key: (1). Sera. (2). Titers and indices of neutralization of sera.
 (3). Viruses. (4). Machupo (Karvallo). (5). Takaribe.

FOOTNOTE 1. In numerator - titer of serum, expressed in \log_2 , in denominator - index of the neutralization of virus, expressed in \log_{10} of the interfering doses. ENDFOOTNOTE.

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Table 5. Detection of antibodies in reconvalescent T after BGL
(case of laboratory contamination).

(1) Ре- ак- ция	(2) Антиген	(3) Титры сывороток									
		(4) Сыворотки реконвалесцента Т.					(5) Контрольные антисыворотки к вирусам.				
		(6) 3 день	(6) 16 день	(6) 40 день	(7) 4 мес.	(7) 7 мес.	C-80/81	(8) Мачупо (CTV 4517)	(9) Хунин (J 793)	(10) Такарибе (TR 11573)	(11) КГЛ (Пятица.)
	T-1610 ¹	0	4	32	16	0	64	64	16	8	0
	C-80/81	0	4	32	16	0	64	128	32	8	0
РСК	Мачупо (CTV 4517) ²	0	4	32	16	0	64	128	32	8	0
(12)	Хунин (HJ 15950)		0	8	4	0	32	32	64	8	0
(13)	Такарибе (TR-11673)		0	4	0		16	32	32	32	0
(14)	КГЛ (Пятицкая)	0	0	0	0		0	0	0	0	64
(15)	Вирус Мачупо (CTV-4517)	0		64	32	8	64	128		8	0
РСК	Вирус Такарибе (TR-11573) ²			0	0	0	0	0		128	0
	T-1610	0		64	32	8	64	64		8	0

Key: (1). Reaction. (2). Antigen. (3). Titters of sera. (4). Sera of reconvalescent T. (5). Control antiserums to viruses. (6). day. (7). mo. (8). Machupo. (9). Hunin. (10). Takaribe. (11). KGL (Pyatnits.). (12). KGL (Pyatnitskaya). (13). Virus of Machupo. (14). Virus of Takaribe.

FOOTNOTE 1. T-1610 - virus, isolated from the blood of the patient T.

2. PH was placed according to method of interference with permanent dose of interfering virus (100 AFD_{50}) and double increasing breedings/cultures/dilutions of serum. ENDFOOTNOTE.

In neutralization reaction the build-up/growth of antibodies in the sera of reconvalescent T is noted only with the strains of virus Machupo (CTV 4517) and T-1610).

The results of serum diagnostics confirm the bond of the disease of the laboratory worker T with Bolivian hemorrhagic fever.

Discussion.

Liberation of the new strains C-80/81, C-58, C-79, and C-84 from the organs/controls of rodents *Calomys callosus* (28.50/o of a quantity of inspected parties/batches). Proving to be identical to the prototype strain of virus Machupo, confirms American researchers' conclusion (K M Johnson et al, 1966) that reservoir and carrier of virus Machupo - the causative agent of Bolivian hemorrhagic fever in nature are the rodents forms/species *Calomys callosus*.

REFERENCES

1. Лещинская Е. В., Чумаков М. П., Мартыненко И. Н., Ткаченко Е. А., О случаях лабораторного заражения боливийской геморрагической лихорадкой. В кн.: Актуальные проблемы вирусологии и профилактики вирусных заболеваний. М., 1972, 325—326.
2. Поваляшина Т. П. Об эпидемиологии боливийской геморрагической лихорадки. Там же, 322.
3. Чумаков М. П., Рубин С. Т., Эльберт Л. Б., Первыков Ю. В. Применение полиэтиленгликоля для концентрации вирусов. 1968. Вopr. вирусол., 6, 721.
4. Johnson K. M., Wiehenga N. H., Mackenzie R. B., Kuns M. Z., Tauraso N. M., Shelokov A., Webb P. A., Justines G., Beye H. K. Virus isolations from human cases of hemorrhagic fever in Bolivia. 1965, Proc. Soc. Exp. Biol. Med., 118, 113—118.
5. Johnson K. M., Kuns M. Z., Mackenzie R. B., Webb P. A., Yunker C. E. isolation Machupo virus wild rodent *Calomys Callosus*. 1966. Am. J. Trop. Med. Hud. 15, 1, 103—106.
6. Buckley S. M., Casals I. Lassa fever a new virus disease of man from West Africa. III isolation and Characteriz of the virus. 1970, Am. J. Trop. Med. Hyg. 19, 4, 680.
7. Murphy F. A., Webb P. A., Johnson K. M., Whitfield S. G. Morphological comparison of machupo with LCM virus basis for a new taxonomic group. 1969, J. Virology. 4, 535—541.
8. Rowe W. P., Murphy F. A., Bergold G. H., Casals I., Hotchin I., Johnson K. M., Lehmann F., Mims C. A., Traub E., Webb P. A. Arenaviruses: proposed name for a newly defined virus group. 1970, J. virol., 5, 651—652.
9. Rowe W. P., Pugh W. E., Webb P. A., Peters C. V. Serological relationship of the Tacaribe complex of viruses to lymphocytic choriomeningitis virus., 1970, J. Virol. 5, 289—292.
10. Speir R. W., Wood O., Liebhaber H., Buckley S. M. Lassa fever a new virus disease of man from west Africa. IV. Electron microscopy of Vero cell cultures infected with Lassa virus. 1970. Am. J. Trop. Med. Hyg. 19, 4, 682—684.

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IMPROVEMENT OF THE SEROLOGICAL IDENTIFICATION OF VIRUS MACHUPO WITH
THE AID OF THE REACTION OF PRECIPITATION IN AGAR GEL AND
NEUTRALIZATION OF VIRUS ACCORDING TO THE METHOD OF INTERFERENCE.

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Summary.

Are developed and approved the procedures of the setting of
reacting the diffusion precipitation in agar and neutralization
reaction, based on the interference of viruses for serological
investigations with Bolivian, Argentinean hemorrhagic fevers and
other infections, called Arena viruses.

Introduction.

The basic serological methods, utilized at present by the
authors during the study of the viruses of group of Takaribe, are the

reaction of the joining of complement (RSK) and the neutralization reaction of platelets in cultures Vero, MA-III, Wi-26 and others (P. A. Webb, 1965; N. H. Wiebuga, 1965; E. A. Webb et al, 1969; L. S. Rhim et al, 1969).

In studying the new strains of virus Machupo, isolated by us in 1971 and their comparisons with the prototype strains of group Takaribe, was possible successfully to apply two, for the first time used for studying these viruses of the procedures, improving serological identification and serum diagnostics.

Materials and methods.

1. Strains of viruses. In work were used the following viruses: Machupo (strain CTV 4517 and C-80/81), Takaribe (strain TR 11573), Hunin (strain HJ 15950), Tamiama (strain SDS^W 10777), Amapari (An 70563), LKhM, KGL (strain Pyatnitskaya), virus of vesicular stomatitis (strain "Indiana").

2. Sera. Specific hyperimmune sera were obtained by the immunization of guinea pigs, white rats, rabbits and adult mice.

3. RSK. Reaction of joining of complement was placed on microtitrator "Takachi" employing conventional procedure, using borate-salt, sugar-acetone and chloroform antigens.

4. RDPA. For setting of reacting diffusion precipitation in gel of agar was used micromethod on microscope slides. Cerebric and cultural antigens for RDPA were prepared according to the method, described by M. P. Chumakov with co-authors (1968), with the aid of concentration by polyethylene glycol. The method of exhaustion consisted of the following: to the undiluted immune serum was added the equal volume of concentrated cerebric antigen, mixture incubated at 37°C 1.5 hours, they centrifuged with 5000 r/min during 15 minutes and precipitation fluid/liquid used as serum for determination in RDPA of the remaining antibodies.

5. Method of interference of viruses. The reaction of interference against the virus of vesicular stomatitis was realized in test-tube cultures of KKE. The procedure of the setting of reacting the interference of viruses is in detail described by E. A. Tkachenko (1970) during the study of virus KGL.

Results.

As can be seen from Table 1, the greatest activity in two

forms/species of serological reactions possessed the cerebriic antigens, concentrated with polyethylene glycol 50 times. However, also the cultural concentrated 300 times antigens are completely suitable for utilization. The reaction of diffusion precipitation in the gel of agar on glass (RDPA), applied by us for the first time for studying the viruses of group Takaribe - LKhM, makes it possible to conduct the differentiation between the separate members of this group (Table 2). In the experiment/experiences of crossed titration both with the homologous ones and with heterologous antigens are determined the titers of antibodies in RSK and RDPA, moreover with the aid of RDPA more clearly are revealed/detected the differences between the individual representatives of group of Takaribe.

It should be noted that we did not succeed in coming to light/detecting/exposing in RSK and RDPA of any degree of the relationship between the antigens of lymphocytic choriomeningitis, which was being considered the prototype of the family of Arena viruses (where was included group Takaribe) and the antigens of group Takaribe. In RSK between the antigens of viruses Tamiami and Machupo revealed one-way communication, at the same time in RDPA antigen Tamiami reacts only with homologous serum. The closest relationship of antigens is established/installed in RSK with viruses Machupo, Hunin, Amapari, and Takaribe.

The use/application of RDPA made it possible to come to light/detect/expose quantitative differences in the antigenic bonds of these four viruses. On data of RDPA, with native antiserums most closely confronting to virus Machupo, proved to be the virus of Hunin, then the viruses of Amapari and Takaribe.

The results of experiment/experiences with the exhaustion of antiserum against virus of Machupo by the concentrated antigens of Hunin, Takaribe, Amapari and KGD (control) are represented in Table 3.

The concentrated antigen of Machupo completely exhausted homologous serum. In combination serum of Machupo + the heterologous antigen of full/total/complete exhaustion did not begin, that can testify about the presence of the species differences between the investigated viruses, including between viruses of Machupo (BGL) and Hunin (AGL).

During the contamination of the primary culture of the cells of chicken/gallinaceous embryos the viruses of Machupo and Takaribe caused interference against the virus of vesicular stomatitis.

[Page 145.] Table 1. Titers of antigens in RSK and RDPA with homologous antisera (depending on the method of preparation of antigens).

(1) Антигены вирусов:	(2) Виды мозговых антигенов								(3) Культуральных антигены			
	(4) боратно-солевой		(5) сахарозо-ацетоновый		(6) хлороформ-мемный		(7) концентрированный ПЭГХ50 боратный		(8) КПЗМ нативный		(9) КПЗ концентр. ПЭГХ300	
	РСК	РДПА	РСК	РДПА	РСК	РДПА	РСК	РДПА	РСК	РДПА	РСК	РДПА
(10) Мачупо (C-8U81)	16	0	64	+ ¹	32	+	256	8	0	0	32	+
(11) Мачупо (CTV 6517)	16	0	64	+	32	+	256	8	0	0	32	+
(11) Такарибе (TR 11673)	16	0	32	+	16	0	256	8	0	0	32	+

Key: (1). Antigens of viruses. (2). Forms/species of cerebri-
 antigens. (3). Cultural antigens. (4). Borate-salt. (5).
 Saccharine-acetone. (6). chloroform. (7). concentrated PEGx50 borate.
 (8). KPZM native. (9). KPZ concentr. of PEGx300. (10). Machupo.
 (11). Takaribe.

FOOTNOTE 1. + antigen reacts in RDPA only in the undiluted
 form/species. ENDFOOTNOTE.

Table 2. RSK-RKPA. Antigen interrelations between viruses in groups Takaribe - LHM (checked titration).

(6) Антигены	(1) Сыворотки		(2) Мачупо (C-80/81)		(3) Такарибе (TR 11573)		(4) Тамнами		(5) Амапари		ЛХМ	
	РСК	РДПА	РСК	РДПА	РСК	РДПА	РСК	РДПА	РСК	РДПА	РСК	РДПА
(2) Мачупо (C-80/81)	64/128	8/16	32/64	+ / 2	0/0	0/0	32/16	2/2	0/0	0/0	0/0	0/0
(3) Такарибе (TR 11573)	16/128	2/+	32/128	8/8	0/0	0/0	16/32	4/2	0/0	0/0	0/0	0/0
(4) Тамнами (CDCW-10777)	32/16	0/0	0/0	0/0	32/32	4/4	0/0	0/0	0/0	0/0	0/0	0/0
(5) Амапари (An 70563)	64/64	2/2	8/16	0/0	0/0	0/0	32/32	4/4	0/0	0/0	0/0	0/0
(7) Хуин (HJ 15950)	32/64	4/8	8/16	0/0	0/0	0/0	32/16	+ / +	0/0	0/0	3/	4/16
ЛХМ	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	22/356			

Key: (1). Sera. (2). Machupo. (3). Takaribe. (4). Tamiami. (5).

Amapari. (6). Antigens¹.

FOOTNOTE 1. In RSK were used saccharine-acetone antigens, in RDPA - borate-salt antigens, concentrated PEGx100. ENDFOOTNOTE.

(7). Huin.

FOOTNOTE 2. In Numerator - titer of the antigen; in denominator - titer of serum.

3. + serum or antigen react in RDPA only in undiluted form/species. ENDFOOTNOTE.

Degree and dynamics of the interfering activity of viruses in culture KKE are represented in Table 4. The maximum of the resistance of cells against VVS is noted to 5-6 days from moment/torque the contamination of culture. The duration of the blocking capacity, i.e., stability of interference, comprises entire period of observation (in this case - 8 days) and is limited only to the onset of the nonspecific degeneration of cells in control cultures.

A phenomenon of the suppression of the interfering activity of viruses we have used successfully in neutralization reaction with the hyperimmune sera of laboratory animals (Table 5) and the sera of reconvalescents after BGL.

Table 3. RDPA. Crossed interrelations in group Takaribe.

Experiment/experience with the exhaustion* of antiserum of Machupo (C-80/81) by the concentrated antigens of Machupo, Hunin, Takaribe, Amanari, KGL (without titration).

(7) Антитела: сыворотка + вирус или без вируса	(1) Антигены в РДПА					
		(2) Мачупо C-80/81	(3) Хуни HJ15950	(4) Такарибе TR11573	(5) Амапари Ap70563	(6) КГЛ Пятницкая (контроль)
(2) Мачупо (C-80/81) + Мачупо (2)		0	0	0	0	0
(3) Мачупо (C-80/81) + Хуни (3)		+	0	0	0	0
(4) Мачупо (C-80/81) + Такарибе (4)		+	+	0	+	0
(5) Мачупо (C-80/81) + Амапари (5)		+	+	0	+	0
(6) Мачупо (C-80/81) + КГЛ (6)		+	+	+	+	0
(7) Мачупо (C-80/81) нативн. (7)		+	+	+	+	0
(8) Такарибе (TR-11573) нативн. (8)		+	0	+	0	0
(9) Амапари (Ap 70563) нативн. (9)		+	+	+	+	0
(9) КГЛ (Пяти.) нативная (контроль)		0	0	0	0	+

Key: (1). Antigens in RDPA. (2). Machupo. (3). Hunin. (4). Takaribe. (5). Amanari. (6). KGL Pyatnitskaya (control). (7). Antibodies: serum + virus or without virus. (8). it is native. (9). KGL (Pyatn.) native (control).

Table 4. Dynamics of interfering activity of viruses Machupo and Takaribe in culture KKE anti.

(1) Вирусы	(2) Титр интерферирующей активности в \log_{10} ИФД ₅₀ /1,0					
	24 час. (2)	48 час. (3)	72 час. (4)	96 час. (5)	144 час. (6)	192 час. (7)
(4) Мачупо (C-80/81)	0	2,0	4,0	5,5	6,5	6,5
(4) Мачупо (CTV 4617)	0	1,5	4,0	6,0	6,5	6,5
(5) Такарибе (TR 11573)	0	1,0	3,5	5,5	7,0	7,0

Key: (1). Viruses. (2). Titer of interfering activity in \log_{10}

IFD₅₀/1.0. (3). hour. (4). Machupo. (5). Takaribe.

Table 5. Neutralization reaction according to the method of interference for studying the interrelations between viruses Machupo, Takaribe and KGL.

(1) Сыворотки (6) Вирусы (100 ИФД ₅₀)	(2) Титры антисывороток			
	(3) С-80/81	(4) Мачупо (CTV 4517)	(5) Такарибе (TR 11673)	(6) КГЛ (Пятницкая)
С-80/81	128	128	8	0
Мачупо (CTV 4517)	64	128	8	0
Такарибе (TR 11673)	0	0	128	0
КГЛ (Пятницкая)	0	0	0	64

Key: (1). Sera. (2). Titters of antisera. (3). Machupo. (4). Takaribe. (5). KGL (Pyatnitskaya). (6). Viruses.

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Discussion.

The reaction of diffusion precipitation in agar, used at present for the serological identification of the series/number of viruses and, for the differentiation of the separate viruses of the complex of the tick-borne encephalitis (S. G. Rubin with co-auth.), in particular, proved to be effective and during the study of representatives from the group of Arena viruses.

In RDPA was possible more clearly than in RSK, to come to light/detect/expose the differences between the separate viruses of subgroup Takaribe.

A phenomenon of the interference of viruses Machupo and Takarabe in culture KKE against the virus of vesicular stomatitis have are used we for the setting of neutralization reaction. With the aid of PH proved to be possible to conduct definitive identification of the newly isolated strains of virus Machupo, and to also apply it for a serum diagnostics with EGL.

REFERENCES

1. Чумаков М. П., Рубин С. Г., Эльберт Л. В., Первилов Ю. В. Применение поли-этиленигликоля для концентрации вирусов. 1968, Вopr. вирусологии, 6, 721.
2. Ткаченко Е. А. Изучение арбовирусов в культурах клеток почек сирийских хомяков. 1970. Автореферат, диссерт. канд. мед. наук.
3. Webb P. A. Properties of Machupo virus. 1965, Am. J. Trop. Med. Hyd., 14, 5, 799.
4. Webb P. A., Johnson K. M., Mackenzie R. The measurement of specific antibodies in Bolivian Hemorrhagic fever in cell cultures. 1969, Proc. Soc. Exp. Biol. Med., 130, 1013—1019.
5. Wiebenga N. H. Immunologi studies on Tacaribe, Iunin and Machupo virus. 1965, Am. J. Trop. Med. Hyd., 14, 5, 799.
6. Rhim I. S., Simizu B., Wiebenga N. H. Multiplication of Machupo virus the etiological agent of Boliwan hemorrhagic fever in cell cultures. 1969. Proc. Soc. Exp. Biol. Med. 130, 2, 382—387.
7. Рубин С. Г., Чумаков М. П., Гавриловская И. Н. Изучение антигенных взаимосвязей между вирусами антигенной подгруппы клещевого энцефалита (КЭ) с применением новой модификации реакции диффузионной преципитации в геле агара (РДПА). 1972. Актуальные проблемы вирусологии и профилактики вирусных заболеваний. 249—250.

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